



Isocitrate dehydrogenase variants in cancer — Cellular consequences and therapeutic opportunities

Shuang Liu^a, Tom Cadoux-Hudson and Christopher J. Schofield

Abstract

Abnormal metabolism is common in cancer cells and often correlates with mutations in genes encoding for enzymes involved in small-molecule metabolism. Isocitrate dehydrogenase 1 (*IDH1*) is the most frequently mutated metabolic gene in cancer. Cancer-associated substitutions in *IDH1* and *IDH2* impair wild-type production of 2-oxoglutarate and reduced nicotinamide adenine dinucleotide phosphate (NADPH) from isocitrate and oxidised nicotinamide adenine dinucleotide phosphate (NADP⁺), and substantially promote the *IDH* variant catalysed conversion of 2-oxoglutarate to D-2-hydroxyglutarate (D-2HG). Elevated D-2HG is a biomarker for some cancers, and inhibition of *IDH1* and *IDH2* variants is being pursued as a medicinal chemistry target. We provide an overview of the types of cancer-associated *IDH* variants, discuss some of the proposed consequences of altered metabolism as a result of elevated D-2HG, summarise therapeutic efforts targeting *IDH* variants and identify areas for future research.

Addresses

Chemistry Research Laboratory, Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA, UK

Corresponding author: Schofield, Christopher J (christopher.schofield@chem.ox.ac.uk)

^a Present address: Broad Institute of MIT and Harvard, 415 Main Street, Cambridge, MA 02142, USA.

Current Opinion in Chemical Biology 2020, 57:122–134

This review comes from a themed issue on **Chemical Genetics and Epigenetics**

Edited by Akane Kawamura and Arasu Ganesan

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 8 August 2020

<https://doi.org/10.1016/j.cbpa.2020.06.012>

1367-5931/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords

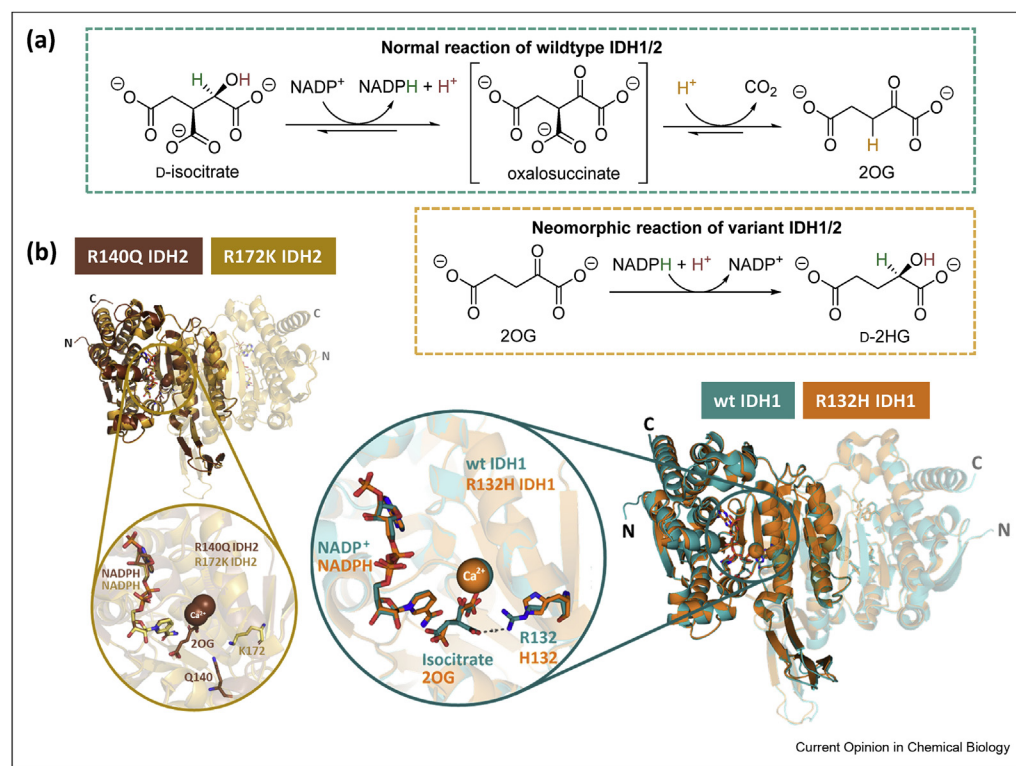
2-Hydroxyglutarate, Isocitrate dehydrogenase, Cancer metabolism, 2-oxoglutarate, Alpha-ketoglutarate, JmJc demethylase, Epigenetics, Hypoxia-inducible factor, Glioma, Acute myeloid leukaemia.

Introduction to *IDH* mutations and cancer

Abnormal metabolism of cancer cells often correlates with mutations in genes encoding for metabolic enzymes, including those involved in the tricarboxylic acid (TCA) cycle and related metabolism, such as succinate dehydrogenase and fumarate hydratase [1]. The isocitrate dehydrogenase 1 (*IDH1*) gene is the most frequently identified mutated metabolic gene in cancer; *IDH1* and *IDH2* mutations cause active site substitutions with consequent profound effects on *IDH* activity, cellular metabolism and cancer development [2–5]. There are three human *IDH* isoforms, that is, the closely related homodimeric *IDH1* and *IDH2* (~70% identity) and the more distantly related heterotetrameric (2 α ,1 β ,1 γ) *IDH3*. *IDH1* localises to the cytoplasm and peroxisomes; *IDH2* and *IDH3* localise to mitochondria. *IDH1* and *IDH2* undergo mutations correlating with >80% of low-grade glioma (LGG) [6] and ~20% of acute myeloid leukaemia (AML) cases [7]. By contrast, no tumour-associated *IDH3* mutations are reported [8]. *IDH3* catalyses the NAD⁺-dependent oxidative decarboxylation of D-isocitrate giving 2-oxoglutarate (2OG) in the TCA cycle, a reaction reported to be irreversible under physiological conditions [9]. *IDH1* and *IDH2* catalyse the reversible oxidised nicotinamide adenine dinucleotide phosphate (NADP⁺)-dependent oxidative decarboxylation of D-isocitrate to 2OG [10], in a manner regulating isocitrate and 2OG levels and which provides reduced nicotinamide adenine dinucleotide phosphate (NADPH) [10]. Cancer-associated substitutions in *IDH1* and *IDH2* impair wild-type (wt) activity—producing 2OG by promoting a ‘neomorphic’ reaction that converts 2OG to D-2-hydroxyglutarate (D-2HG), using NADPH as a cosubstrate [11] (Figure 1a).

The nature of *IDH* substitutions varies with the cancer type; in many cancers *IDH* mutations are rare or not observed; the reasons for these differences are unclear [4,5]. In AML, for example, *IDH* substitutions are common, whereas with multiple myeloma, another blood cancer, they are rare. In LGG, the majority (>80%) of *IDH* mutations occur in the *IDH1* gene, being dominated by R132H *IDH1* [15]. Less frequently, substitutions occur at *IDH2* R172 [6,16], which is located at a structurally

Figure 1



Reactions catalysed by wild-type (wt) and variant isocitrate dehydrogenases. (a) Oxidative and reductive reactions catalysed by wt and variant IDH1/2, respectively. The reversible conversion of isocitrate to 2OG and CO₂ by wt IDH1/2 proceeds via NADP⁺-mediated oxidation of isocitrate giving unstable oxalosuccinate, which undergoes β -keto decarboxylation giving 2OG. IDH1/2 variants catalyse reduction of 2OG to D-2HG using NADPH. IDH reactions require Mg²⁺/Mn²⁺ [11]. (b) Overall and expanded active site views from crystal structures of wt IDH1 (teal, PDB 1T0L) [12], R132H IDH1 (orange, PDB 3INM) [11], R140Q IDH2 (brown, PDB 5I95) [13] and R172K IDH2 (gold, PDB 5SVN) [14]. One monomer in the homodimer is differentiated by a different transparency level. Each active site is bound to a cofactor (NADP⁺ for wt IDH1; NADPH for R132H IDH1, R140Q IDH2 and R172K IDH2) and a substrate (isocitrate for wt IDH1; 2OG for R132H IDH1 and R140Q IDH2) and an inhibitory Ca²⁺ (positioned to coordinate to the substrate). 2OG, 2-oxoglutarate; IDH, isocitrate dehydrogenase; wt, wild-type.

analogous position to IDH1 R132 (Figure 1b). This contrasts with AML where *IDH2* mutations occur at a similar or higher frequency compared with *IDH1* mutations [15]. The most common IDH substitution in AML is IDH2 R140Q. The analogous IDH1 R100Q variant is rarer, being only found in grade II/III gliomas [17,18]. Interestingly, *IDH1* and *IDH2* mutations appear to be mutually exclusive [19]. All the substituted arginine residues (IDH1 R132/R100 and IDH2 R172/R140) are likely directly or indirectly involved in binding isocitrate and 2OG at the IDH1/2 active sites [12] (Figure 1b). The precise details of how substitutions impact on the individual steps of the complex Mg²⁺-using IDH mechanisms are unclear.

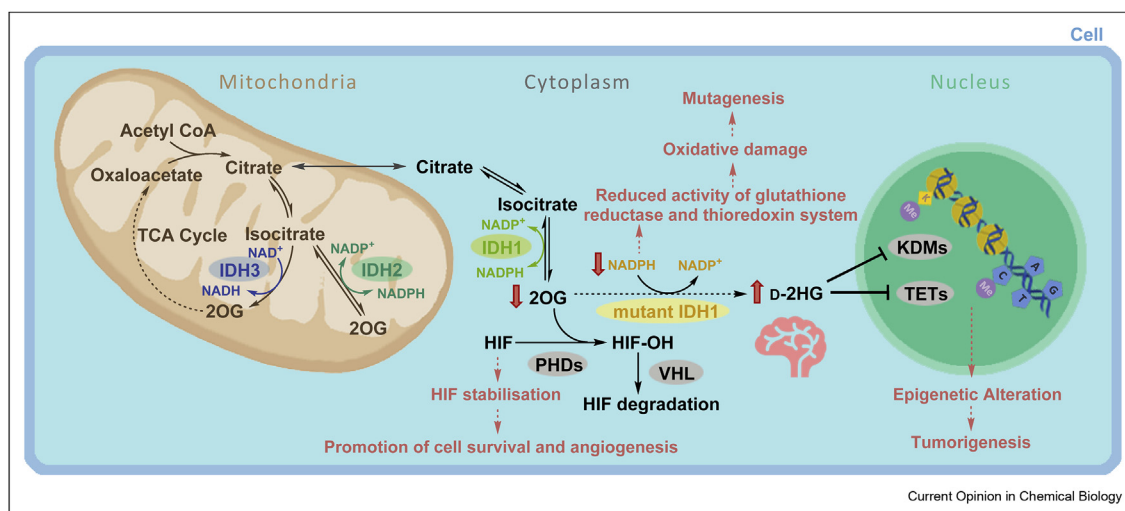
The metabolic consequences of IDH mutations

Elevated D-2HG levels

Amongst the multifaceted cellular impacts of *IDH* mutations in malignancies (Figure 2), the substantially increased levels of D-2HG stand out, leading to its

description as an ‘oncometabolite’ and the proposal that elevated D-2HG levels promote tumorigenesis [20]. Studies using metabolomics mass spectrometry analyses demonstrated that the D-isomer of 2HG ((R)-2HG) accumulates in >100-fold excess relative to normal levels in cells/tissues of patients with LGG and AML, harbouring *IDH1/2* mutations [11,21,22]. Most, but not all, studies report a less substantial 2OG reduction, with other TCA cycle intermediate levels being relatively unchanged [23]. Although variant IDHs consume 2OG, cellular 2OG stocks can be replenished from other sources, including glutamine [24]. On the other hand, whilst D-2HG produced in normal cells (where its roles are unclear) can be cleared by D-2HG dehydrogenase (D2HGDH) catalysed conversion to 2OG, it seems the normal clearance rate of D2HGDH is insufficient to suppress the high levels of D-2HG produced in IDH variant-bearing cells [11,25]. The mitochondrial localisation of D2HGDH might further contribute to its ineffectiveness in clearing cytosolic D-2HG produced by IDH1 variants [25].

Figure 2



Roles of wt IDH1/2/3 and some of the potential multiple effects of IDH mutation in cells (exemplified for IDH1). Different subcellular localisations (IDH1: cytoplasm; IDH2/3: mitochondria) and cosubstrate usage (IDH1/2: NADP⁺; IDH3: NAD⁺) distinguish the 3 human IDHs. The effects of IDH1 variants, including promotion of tumorigenesis, are proposed to manifest because of metabolic changes including D-2HG accumulation, depletion of NADPH and/or reduced 2OG. Changes in D-2HG/2OG levels are proposed to inhibit 2OG oxygenases involved in regulation of expression, for example, PHD, JmJC KDM, or TET enzymes. 2OG, 2-oxoglutarate; D-2HG, D-2-hydroxyglutarate; IDH, isocitrate dehydrogenase; HIF, hypoxia-inducible factor; HIF-OH, hydroxylated HIF; PHD, HIF prolyl hydroxylase domain enzyme; KDM, histone lysine demethylase; NADP⁺, oxidised nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; TCA, tricarboxylic acid; TET, ten-eleven translocation oxygenase; VHL, Von Hippel-Lindau; wt, wild-type.

Elevated D-2HG levels serve as a robust biomarker for *IDH1/2* mutations in gliomas [26]. D-2HG levels in plasma/serum can be analysed by liquid chromatography-mass spectrometry (LC-MS) and in the case of gliomas, D-2HG can be imaged by magnetic resonance spectroscopy [27,28]. Antibodies for IDH variants, in particular for IDH1 R132H, are also potentially useful for diagnosis. Aside from diagnosis and medicinal chemistry opportunities, the discovery of the elevated D-2HG levels was exciting from a cancer biochemistry perspective, because it opened opportunities to link readily quantifiable (*in vitro* and *in vivo*) changes in the levels of a specific metabolite (D-2HG), with cellular processes relevant to cancer, such as tumorigenesis and epigenetic regulation.

2-oxoglutarate-dependent oxygenases

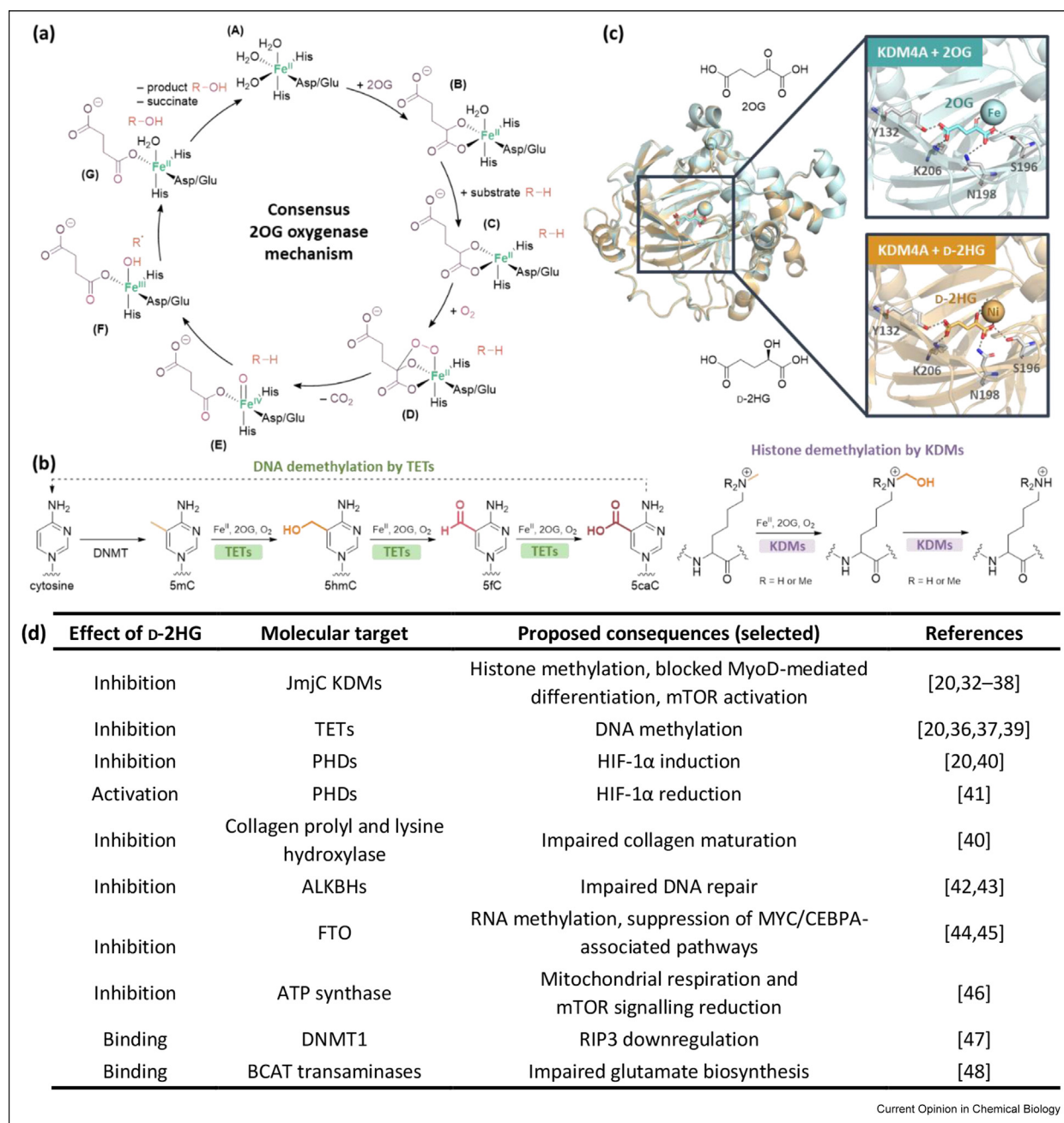
Before the work on IDH variant neomorphic activity, cancer-linked loss-of-function mutations to TCA cycle enzymes other than IDH, succinate dehydrogenase and fumarate hydratase, were identified [29]. The consequently elevated levels of succinate and/or fumarate are proposed to inhibit human 2OG and Fe(II) dependent oxygenases [30]. Given the structural similarity between D-2HG and 2OG, it was also proposed that elevated D-2HG levels competitively inhibit 2OG oxygenases in manner relevant to cancer (Figure 3c). 2OG oxygenases couple two electron substrate

oxidations, typically hydroxylation or demethylation via hydroxylation, to the conversion of 2OG and dioxygen to succinate and carbon dioxide (Figure 3a). Human 2OG oxygenases have roles in collagen biosynthesis, lipid metabolism, DNA/RNA damage repair/modification, ribosomal/translation machinery modification, the hypoxic response, and epigenetics/chromatin biology [31].

It is proposed that elevated levels of D-2HG competitively inhibit 2OG oxygenases involved in epigenetic regulation, including the Jumonji C domain-containing histone lysine demethylases (JmJC KDMs) and the ten-eleven translocation (TET) oxygenases, which regulate expression by catalysing histone demethylation and oxidation of *N*-methylcytosine in DNA, respectively (Figure 3b,d) [20,32–39]. Competitive inhibition of the JmJC KDMs and the TETs by D-2HG could contribute to the histone and DNA hypermethylation states manifested in IDH variant gliomas and AML [20,32–35,39,50]. Such chromatin states are proposed to block differentiation of (potential) cancer cells, enabling them to grow and proliferate, thereby promoting tumorigenesis [51]. Figure 3d summarises the diverse cellular consequences proposed to result from elevated levels of D-2HG.

The combined studies indicate that the effects of D-2HG on chromatin are potentially complex, though in

Figure 3



Current Opinion in Chemical Biology

2-hydroxyglutarate may inhibit 2-oxoglutarate oxygenases in a cancer-relevant manner. (a) Consensus mechanism for the 2OG oxygenases. The substrate is hydroxylated in the presence of Fe(II), 2OG and O₂, giving succinate and CO₂ as by-products. (b) Examples of reactions catalysed by 2OG oxygenases involved in chromatin regulation, as catalysed by TETs and JmjC KDM methyl-group modifying enzymes; DNA cytosine demethylation is catalysed by TETs and histone lysine demethylation catalysed by the JmjC KDMs. (c) Views from crystal structures of the JmjC KDM4A in complex with 2OG or D-2HG showing their analogous binding modes. Both 2OG (pale teal, PDB 2YBK [32]) and D-2HG (pale orange, PDB 2GP5, half maximal inhibitory concentration [IC₅₀] against KDM4A = 24 μM [49]) occupy the same binding site and interact with KDM4A Y132, S196, N198 and K206. (d) Some of the multiple cellular targets and pathways potentially affected by D-2HG accumulation. There is mixed evidence on whether the mammalian target of rapamycin (mTOR) [35,46] and hypoxia-inducible factor-1α (HIF-1α) [20,40,41] are activated by D-2HG [42–45,47]. ALKBH, 2OG-dependent AlkB homologue; 2OG, 2-oxoglutarate; BCAT, branched-chain amino acid transferase; CEBPA, CCAAT enhancer binding protein alpha; D-2HG, D-2-hydroxyglutarate; DNMT1, DNA methyltransferase 1; FTO, fat mass and obesity-associated protein; HIF, hypoxia-inducible factor; HIF-OH, hydroxylated HIF; IDH, isocitrate dehydrogenase; JmjC KDM, Jumonji C domain-containing histone lysine demethylase; KDM, histone lysine demethylase; mTOR, the mammalian target of rapamycin; PHD, HIF prolyl hydroxylase domain enzyme; RIP3, receptor-interacting protein 3; TET, ten-eleven translocation oxygenase [42–45,47,83–89].

some contexts dysregulation of specific enzymes may be particularly important, leading to candidate medicinal chemistry targets. It should be noted that D-2HG is not a potent inhibitor of, at least many, 2OG-dependent oxygenases, even though its high concentrations in (specific regions of) tumour cells may compensate for its weak inhibition against isolated enzymes.

IDH variants and regulation of hypoxia-inducible factors

Hypoxia-inducible factor (HIF) is an α,β -heterodimeric transcription factor that is a central regulator of the chronic human hypoxic response. HIF is reported to be elevated in multiple types of cancer, in a manner often, but not always, associated with hypoxia and/or mutations to TCA cycle enzymes, including succinate dehydrogenase and IDHs [52]. Both the levels and transcriptional activity of HIFs (human HIF-1 α , -2 α and -3 α) are directly regulated by 2OG oxygenases. Catalysis by the HIF α prolyl hydroxylase domain enzymes (human HIF prolyl hydroxylase domain enzyme [PHD] 1–3) signals for HIF α degradation in an oxygen availability–limited manner, a property enabling them to act as hypoxia sensors (Figure 2). The factor inhibiting HIF, a JmjC HIF α asparaginyl hydroxylase, regulates HIF transcriptional activity by limiting its interactions with transcriptional coactivators [53].

There is mixed evidence on the effects of D-2HG on HIF activity in IDH variant gliomas. Some studies show no correlation between HIF levels and IDH variant gliomas [54,55]; others show either upregulation [40,56] or downregulation [41] of HIF-1 α in cells expressing mutant *IDH1*, possibly reflecting variations in O₂ availability in different studies. It is proposed that microvascular proliferation in glioblastoma multiforme is in part due to vascular endothelial growth factor upregulation due to increased HIF [57]. In a perhaps counterintuitive mechanism for HIF downregulation, PHD catalysis is proposed to be coupled to D-2HG oxidation, that is, D-2HG behaves as a PHD agonist rather than an inhibitor; however, the mechanism(s) underlying the cellular observations is unclear because other studies do not find D-2HG to be a PHD substrate [41,58]. Note that the reported half maximal inhibitory concentration (IC₅₀) of D-2HG for PHD2 is high (~ 7.3 mM [32]), and D-2HG concentration in *IDH* mutant glioma is 5–35 mM [11]. Thus, pathophysiologically relevant PHD inhibition by D-2HG is possible, but if this level of inhibition is biologically relevant one might expect multiple enzymes to be inhibited causing cytotoxicity. One possible reason for the cellular observations concerning the agonist activity of D-2HG is its nonenzymatic or enzymatic conversion to 2OG [58], although whether this can occur at sufficient levels is unclear. HIF is often

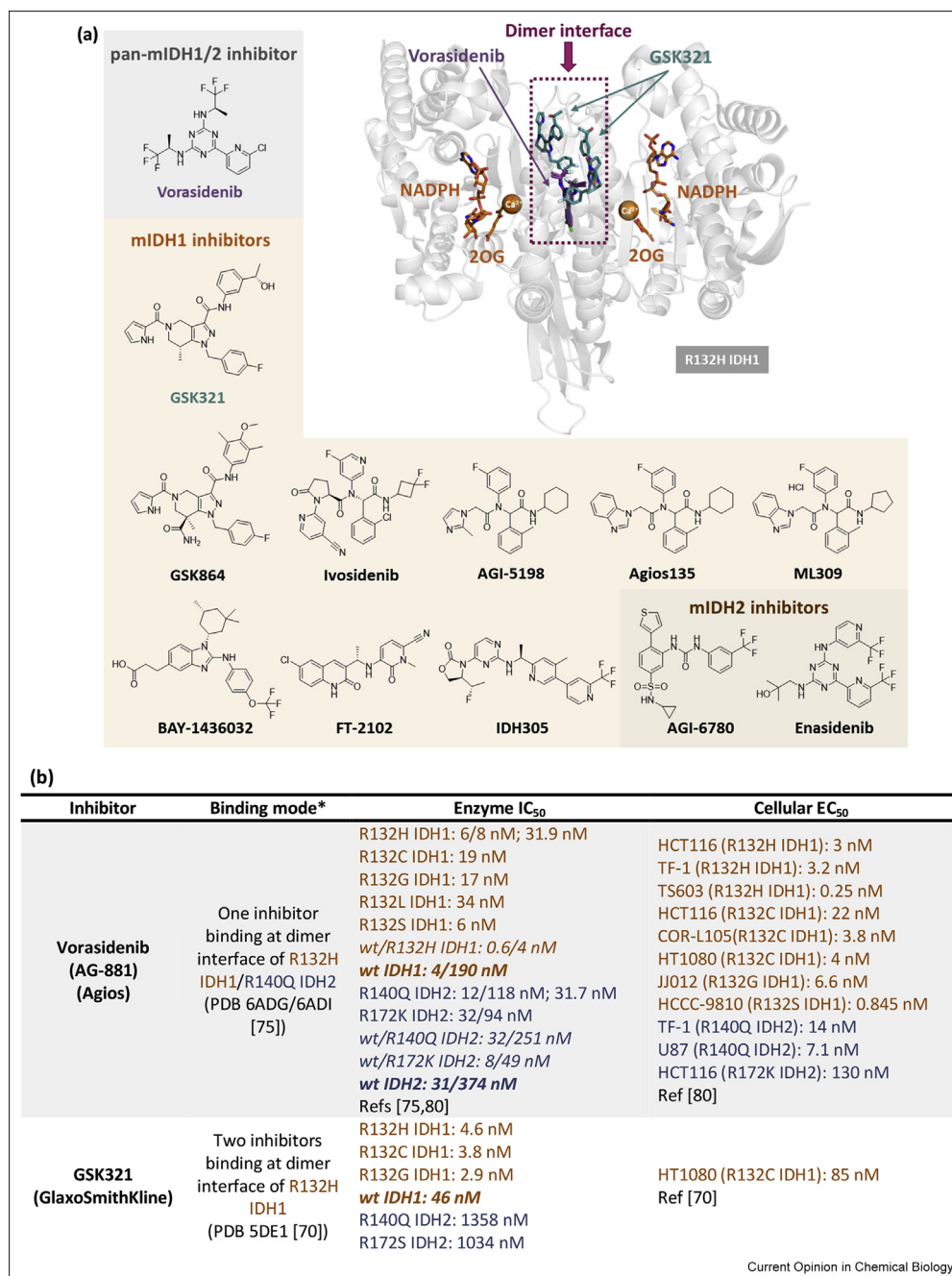
viewed as an oncogene (or proto-oncogene) that is predicted to be activated in cancers and HIF-1 α – and HIF-2 α – suppressing drugs are being pursued [59]. However, some studies suggest that, at least in particular contexts, HIF- α has a tumour suppressor role, including in glioma [60] and leukaemic [61] cells. For example, inhibition of HIF-2 α reduces angiogenesis but enhances tumour growth in glioma cells, partly because of a reduction in tumour cell apoptosis [60]. The expression levels of many HIF target genes are also decreased in *IDH* mutant gliomas [62]. Collectively, these findings raise the possibility that HIF activation via PHD inhibition, by direct or indirect means, may impair mutant *IDH* tumour growth [41].

IDH variants and alterations in the NADPH/NADP⁺ ratio

Mutations in *IDH* genes result in a reduction in the cellular NADPH/NADP⁺ ratio. IDH1 and IDH2 are important sources of NADPH in the cytoplasm and mitochondria, respectively [63]. The overall neomorphic conversion of isocitrate to D-2HG does not necessarily directly change the NADP⁺/NADPH ratio, whereas the normal forward IDH reactions produce NADPH for use in fatty acid biosynthesis and protection against oxidative damage; hence *IDH* mutant–bearing cells may be unusually sensitive to redox-related damage [40,64,65]. One study suggests that the R132H substitution in IDH1 results in $\sim 38\%$ reduction of its NADPH production capacity in glioblastoma tissue samples [64]. Consumption of NADPH for D-2HG synthesis is reported to decrease NADPH-dependent fatty acid synthesis, thereby increasing pentose phosphate pathways to support the NADPH demands and sensitising *IDH1* mutant cells to oxidative stress [66].

Depletion of NADPH also impairs regeneration of the thiol form of glutathione (GSH) (γ –glutamyl-cysteinylglycine) from its disulfide form; GSH is a thiol-containing reducing agent which protects cells from oxidative stress by neutralising reactive oxygen species (ROS). GSH is present at lower levels in both R132H IDH1 overexpressing glioma cells [65] and knock-in mice [40]. Reduction in NADPH and GSH levels can result in oxidative damage, which may contribute to mutations, ultimately promoting tumorigenesis [67]. The reduction in GSH may be exacerbated by depletion of glutamate (a GSH component) in *IDH* mutant–bearing cells [24], where glutamine is converted to glutamate and then to 2OG to replenish its role in the TCA cycle. In support of this, isotope-labelling experiments indicate that D-2HG produced by variant IDH1/2 is derived not only from glucose, but also from glutamine via glutamate [11,66].

Figure 4



Summary of selected variant IDH1 and IDH2 inhibitors. (a) Structures of variant IDH (mIDH) inhibitors and a crystallography-derived representation of how two allosteric inhibitors (superimposed) bind at the dimer interface of R132H IDH1. Vorasidenib (purple, PDB 6ADG [75]) and GSK321 (teal, PDB 5DE1 [70]) have binding stoichiometries of 1 and 2 inhibitors per R132H IDH1 dimer, respectively. The dimer interface where most reported variant IDH inhibitors bind is indicated by the dotted magenta region and is proximate to the active site (Ca²⁺ is inhibitory). **(b)** Binding modes, biochemical half maximal inhibitory concentration (IC₅₀) and cellular half maximal effective concentration (EC₅₀) values of variant IDH inhibitors. Note: Different IC₅₀ values may be (partly) attributed to the different enzyme concentrations/assay conditions. “/” in IC₅₀ values refers to measurements from different incubations times from the same report; “,” separates values from different reports. “wt/mutant” refers to a heterodimer, with other entries representing a homodimer. Data for IDH1 and IDH2 are shown in brown or navy respectively [83–89]. NR: not reported. *As observed by crystallography except where “cryo-EM” is stated. 2OG, 2-oxoglutarate; IDH, isocitrate dehydrogenase; wt, wild-type [42–45,47,83–89].

<p>GSK864 (GlaxoSmithKline)</p> <p>Ivosidenib (AG-120) (Agiros)</p> <p>AGI-5198 (Agiros)</p> <p>Agiros135 (Agiros)</p> <p>ML309 (NCATS/Agiros)</p> <p>BAY-1436032 (Bayer)</p>	<p>NR</p> <p>NR</p> <p>NR</p> <p>NR</p> <p>Cryo-EM: One inhibitor binding at the dimer interface of R132C IDH1 (PDB 5K11 [72])</p> <p>Compound 1 (analogue of BAY-1436032): One inhibitor binding at dimer interface of R132H IDH1 (PDB 5LGE [71])</p>	<p><i>wt IDH2: 496 nM</i> Ref [70]</p> <p>R132H IDH1: 15.2 nM; 0.16 μM R132C IDH1 : 8.8 nM; 0.10 μM R132G IDH1 : 16.6 nM <i>wt IDH1: 466.5 nM; 2.74 μM</i> R140Q IDH2: 1916 nM R172Q IDH2: 22 nM R172S IDH2: 997 nM <i>wt IDH2: 1360 nM; >30 μM</i> Refs [70,83]</p>	<p>U87 (R132H IDH1): 191 nM THP1 (R132H IDH1): 120 nM HT1080 (R132C IDH1): 320 nM; 299 nM SNU1079 (R132C IDH1): 341 nM JJ012 (R132G IDH1): 519 nM RBE (R132S IDH1): 532 nM Refs [70,83]</p>
		<p>R132H IDH1: 12 nM; 0.04 μM R132C IDH1 : 13 nM; 0.05 μM R132G IDH1: 8 nM R132L IDH1: 13 nM R132S IDH1: 12 nM <i>wt/R132H IDH1: 5/12 nM</i> <i>wt IDH1: 24/71 nM; 4.26 μM</i> R172Q IDH2: >30 μM <i>wt/R172K IDH2: 72 μM</i> <i>wt IDH2: >30 μM</i> Refs [82,83]</p>	<p>U87 (R132H IDH1): 19 nM; 50 nM THP1 (R132H IDH1): 19 nM HT1080 (R132C IDH1): 8 nM; 36 nM COR-L105 (R132C IDH1): 15 nM SNU1079 (R132C IDH1): 46 nM JJ012 (R132G IDH1): 16 nM HCCC-9810 (R132S IDH1): 12 nM RBE (R132S IDH1): 220 nM Refs [82,83]</p>
		<p>R132H IDH1: 0.07 μM; 0.39 μM R132C IDH1: 0.16 μM; 1.28 μM <i>wt IDH1: >100 μM; >30 μM</i> R140Q IDH2: >100 μM R172K IDH2: >100 μM R172Q IDH2: >30 μM <i>wt IDH2: >100 μM; >30 μM</i> Refs [83–85]</p>	<p>U87 (R132H IDH1): 0.07 μM; 43 nM THP1 (R132H IDH1): 52 nM HT1080 (R132C IDH1): 0.48 μM; 1.2 μM SNU1079 (R132C IDH1): 1.5 μM JJ012 (R132G IDH1): 1.6 μM RBE (R132S IDH1): 2.0 μM Refs [83,85]</p>
		<p>R132H IDH1: 42 nM; 0.38 μM R132C IDH1: 4 nM; 0.09 μM <i>wt/R132H IDH1: 80 nM</i> <i>wt IDH1: 2.00 μM; 15.6 μM</i> R140Q IDH2: >10 μM R172K IDH2: >10 μM R172Q IDH2: >30 μM <i>wt IDH2: >10 μM; >30 μM</i> Refs [74,83]</p>	<p>HEK293 (R132H IDH1): 81.5 nM U87 (R132H IDH1): 217 nM THP1 (R132H IDH1): 212 nM SNU1079 (R132C IDH1): 480 nM HT1080 (R132C IDH1): 530 nM JJ012 (R132G IDH1): 681 nM RBE (R132S IDH1): 810 nM Refs [74,83]</p>
		<p>R132H IDH1: 96 nM; 0.34 μM R132C IDH1: 62 nM; 0.09 μM <i>wt IDH1: 36 μM; 20.9 μM</i> R172Q IDH2: >30 μM <i>wt IDH2: >30 μM</i> Refs [83,86]</p>	<p>U87 (R132H IDH1): 150 nM; 248 nM THP1 (R132H IDH1): 238 nM SNU1079 (R132C IDH1): 541 nM HT1080 (R132C IDH1): 623 nM JJ012 (R132G IDH1): 711 nM RBE (R132S IDH1): 970 nM Refs [83,86]</p>
		<p>R132H IDH1: 15 nM R132C IDH1: 15 nM <i>wt IDH1: 20 μM</i> <i>wt IDH2: >100 μM</i> Ref [71]</p>	<p>LN229 (R132H IDH1): 73 nM HCT116 (R132H IDH1): 47 nM NCH551b (R132H IDH1): 13 nM HT1080 (R132C IDH1): 135 nM Mouse hematopoietic: R132H IDH1: 60 nM R132C IDH1: 45 nM Human AML patient-derived: R132H IDH1: 5 nM R132C IDH1: 5 nM R132G IDH1: 4 nM R132L IDH1: 3 nM</p>

Current Opinion in Chemical Biology

Figure 4

			R132S IDH1: 16 nM Refs [71,87]
IDH305 (Novartis)	Three inhibitors binding at dimer interface of R132H IDH1 (PDB 6B0Z [73]) IDH889 (analogue of IDH305): two inhibitors binding at dimer interface of R132H IDH1 (PDB 5TQH [88])	R132H IDH1: 18/27 nM R132C IDH1: 28 nM wt IDH1: 6.14 μM Ref [73]	HCT116 (R132H IDH1): 24 nM HCT116 (R140Q IDH2): 3.8 μM HCT116 (R172K IDH2): 10 μM Ref [73]
FT-2102 (Olutasidenib) (Forma)	Two inhibitors binding at dimer interface of R132H IDH1 (PDB 6U4J [76])	R132H IDH1: 21.2 nM R132C IDH1: 114 nM wt IDH1: 22.4 μM R140Q IDH2: >100 μM R172K IDH2: 27.3 μM Ref [76]	HCT116 (R132H IDH1): 21 nM U87 (R132H IDH1): 9 nM HCT116 (R132C IDH1): 94 nM U87 (R132C IDH1): 39 nM U87 (R132G IDH1): 6 nM U87 (R132L IDH1): 42 nM U87 (R132S IDH1): 9 nM Ref [76]
AGI-6780 (Agios)	One inhibitor binding at dimer interface of R140Q IDH2 (PDB 4JA8 [77])	R132H IDH1: 11 μM; 14 μM R132C IDH1: 9.7 μM wt IDH1: >100 μM; >30 μM R140Q IDH2: 23/170 nM R172Q IDH2: 9 nM wt/R140Q IDH2: 4/120 nM wt IDH2: 190/2700 nM; 12 μM Refs [77,83]	U87 (R132H IDH1): >100 μM TF-1 (R140Q IDH2): 18 nM U87 (R140Q IDH2): 11 nM Ref [77]
Enasidenib (AG-221) (Agios)	One inhibitor binding at dimer interface of R140Q IDH2 (PDB 5I96 [13])	R132H IDH1: 48/78 μM; 4.95 μM; 16 μM R132C IDH1: 16 μM wt/R132H IDH1: 0.677 μM wt IDH1: 0.45/1.12 μM; 15 μM R140Q IDH2: 0.1/0.32 μM; 0.77 μM; 0.009 μM R172K IDH2: 0.2/0.4 μM; 0.21 μM R172Q IDH2: 44 nM R172S IDH2: 0.155 μM wt/R140Q IDH2: 0.03/0.31 μM wt/R172K IDH2: 0.01/0.11 μM wt IDH2: 1.8/40 μM; 34 μM; >30 μM Refs [13,80,83,89]	U87 (R132H IDH1): >3 μM HT1080 (R132C IDH1): >3 μM TF-1 (R140Q IDH2): 0.02 μM; 0.012 μM U87 (R140Q IDH2): 0.01 μM; 0.012 μM HCT116 (R172K IDH2): 0.53 μM TF-1 (R172K IDH2): 0.98 μM U87 (R172K IDH2): 1.59 μM; 1.4 μM SW1353 (R172S IDH2): 2.1 μM Refs [13,89]

Current Opinion in Chemical Biology

(Continued).

IDH variants and branched-chain amino acid transferase-1

A related link between *IDH1* mutant cells and amino acids, concerns branched-chain amino acid transferase-1 (BCAT1) which is a 2OG-dependent enzyme catalysing the transamination of branched-chain amino acids (valine, leucine and isoleucine) with 2OG giving glutamate and branched-chain α -ketoacids. In glioma, *IDH1* mutations correlate with lower levels of BCAT1 [68]; D-2HG is also reported to directly inhibit BCAT1, although only weakly [48]. The impairment of BCAT1 catalysis, however, has an impact on cellular metabolism,

in particular, an increase in branched-chain amino acid levels and a decrease in glutamate levels [48]. Levels of other amino acids and other metabolites, including lipids, are reported to be changed in *IDH* mutant-bearing cells, although results are sometimes conflicting and the disease relevance of these changes are unclear.

Therapeutic advances with variant IDH1/2 inhibitors

Following the identification of *IDH* mutations in gliomas and AML, multiple drug discovery campaigns targeting variant IDH1/2 were initiated. The inhibitors developed

can successfully reduce D-2HG levels as shown by studies in cells and animals [69]. The majority of potent ($IC_{50} \leq 100$ nM) R132H IDH1 inhibitors for which crystal structures are available inhibit via an allosteric mechanism, involving binding at the dimer interface, instead of the more typical active-site binding mode. This is interesting given the structural diversity in the allosteric inhibitors [14,70–73] (Figure 4a). The allosteric inhibition is proposed to involve disruption of the binding of catalytically required metal ion (Mg^{2+} or Mn^{2+}) at the active site [74]. Crystallographic data for ivosidenib and analogues (AGI-5198, Agios135, ML309) is lacking, although cryogenic electron microscopy (cryo-EM) data for ML309 [72] suggest it binds at the dimer interface like other allosteric inhibitors (vorasidenib [75], GSK321 [70], BAY-1436032 [71], FT-2102 [76], Novartis 305 [73], AGI-6780 [77], enasidenib [13]) (Figure 4b).

Ivosidenib [78] and enasidenib [79], which target variant IDH1 and IDH2, respectively, received FDA approval for AML treatment. Ivosidenib is in ongoing clinical trials for glioma treatment among other malignancies, in some cases as a combination therapy, for example, with vorasidenib (NCT03343197) and nivolumab (NCT04056910). Enasidenib is in clinical trials mostly for AML and haematological malignancies, including in combination therapy with azacitidine (NCT03683433). Vorasidenib is the only reported inhibitor that targets both variants of IDH1 and IDH2 [80]. Given its blood–brain barrier penetrating ability [80], vorasidenib is promising for glioma treatment, and it is currently in a phase 3 clinical trial for residual and recurrent grade 2 glioma (NCT04164901) and a phase 1 clinical trial for advanced solid tumours including gliomas (NCT02481154). BAY-1436032 has completed a phase 1 clinical trial for advanced AML (NCT03127735) and is currently in a phase 1 clinical trial for advanced solid tumours (NCT02746081). FT-2102 is in phase 1/2 clinical trials for advanced solid tumours and gliomas (NCT03684811), AML and myelodysplastic syndrome (MDS) (NCT02719574). Similarly, IDH305 is in a phase 1 clinical trial for advanced malignancies including gliomas, AML/MDS (NCT02381886); unfortunately, dose-limiting toxicities appear to have halted its clinical development [81]. One series developed by GlaxoSmithKline (e.g. GSK321 and the more bioavailable analogue GSK864) shows low selectivity between wt and variant IDH1 [70], potentially hindering its clinical development—although low wt/variant selectivity is also observed for vorasidenib and ivosidenib (Figure 4b). Preclinical compounds including AGI-5198 and AGI-6780 serve as useful tool compounds but lack clinical applications because of poor metabolic stability [82] and lack of an *in vivo* response, respectively.

Conclusions

The breakthrough discovery that cancer-linked mutations to *IDHs* cause major metabolic changes, notably increased D-2HG, has opened up exciting new therapeutic and diagnostic possibilities. It also provided an opportunity for research to connect *in vitro* and *in vivo* small-molecule biochemistry with the pathophysiology of cancer. From an IDH-variant drug development perspective, work has progressed rapidly with compounds approved for use in AML. Resistance to ivosidenib and enasidenib in the form of a second mutation at the IDH1/2 dimer interface has emerged [90], potentially compromising the long-term efficacy of similar IDH variant inhibitors. It is, however, important to state that the optimal patient populations for deployment of mutant IDH inhibitors have likely not yet been identified. There is also scope for developing new types of IDH inhibitor, including molecules that target the active site, which might manifest reduced resistance compared with the current allosteric type inhibitors.

At least in model systems, it is also of interest to explore inhibition of wt IDH, for which no (selective) inhibitors has been developed. In part, this is because nearly all reported cancer-linked *IDH* gene mutations are heterozygous [6,91], and both the variant homodimer and the wt/variant heterodimer of IDH1 can generate D-2HG. For heterozygous *IDH1* mutant tumours, a significantly lower D-2HG level is found in gliomas that undergo loss of the wt *IDH1* allele [91]. Thus, at least in some circumstances, inhibiting the remaining wt *IDH1* allele to reduce local 2OG availability may alleviate protumour effects of *IDH1* mutations. Wild-type IDH inhibitors are also of interest because there is evidence TCA cycle disruption holds promise for cancer treatment, for example, via inhibition of the 2OG dehydrogenase complex, which converts 2OG to succinyl CoA [92].

Despite the rapid progress in the *IDH* mutant field important basic questions remain. These include the definition of exactly how *IDH* mutations promote tumorigenesis. Studies to date have highlighted the potential of D-2HG to compete with 2OG in its role as a cosubstrate for enzymes involved in epigenetic/transcriptional regulation and metabolism. However, small molecules other than D-2HG may well be involved, and the role of altered metabolism in tumorigenesis and cancer progression is likely context dependent. Although it has not shown to be relevant in humans, the recent discovery that the lysine metabolite 2-oxoadipate can be converted into D-2HG via oxygenase catalysis in bacteria reveals the potential for discovery of new metabolic processes relating to 2OG/2HG and cancer metabolism [93].

One interesting observation is that glioma patients with *IDH1/2* mutations are associated with an increase in overall survival compared with those with wt *IDH* genes [6,64,94]. Consistent with the observations for human gliomas, mouse models expressing R132H *IDH1* manifest increased median survival [95]; in addition to increased D-2HG production, they manifest increased DNA cytosine methylation and reduced infiltration of immune cells [96]. By contrast, wt *IDH1* gliomas correlate with high levels of chemokines and interleukins that stimulate infiltration of immune cells, consistent with poor prognosis [96,97]. Other studies attribute improved prognosis of *IDH* mutant gliomas to their higher sensitivity to chemotherapy [65] and radiotherapy [98]. This may be due to the R132H *IDH1*–induced depletion of NADPH and GSH and/or increased reactive oxygen species generation. These observations highlight the need for detailed context-dependent studies on the biochemistry of tumorigenesis and subsequent events in cancer progression.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

CJS thanks Cancer Research UK and the Wellcome Trust for funding. SL thanks the Agency for Science, Technology and Research (A*STAR, Singapore) for a National Science Scholarship. The authors apologise for incomplete citations due to the focused nature of this short review.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- * of special interest
- ** of outstanding interest

1. Anderson NM, Mucka P, Kern JG, Feng H: **The emerging role and targetability of the TCA cycle in cancer metabolism.** *Protein Cell* 2018, **9**:216–237.
2. Oermann EK, Wu J, Guan KL, Xiong Y: **Alterations of metabolic genes and metabolites in cancer.** *Semin Cell Dev Biol* 2012, **23**: 370–380.
3. Dietlein F, Weghorn D, Taylor-Weiner A, Richters A, Reardon B, Liu D, Lander ES, Van Allen EM, Sunyaev SR: **Identification of cancer driver genes based on nucleotide context.** *Nat Genet* 2020, **52**:208–218.
4. Tommasini-Ghelfi S, Murnan K, Kouri FM, Mahajan AS, May JL, Stegh AH: **Cancer-associated mutation and beyond: the emerging biology of isocitrate dehydrogenases in human disease.** *Sci Adv* 2019, **5**:eaaw4543.
5. Molenaar RJ, Maciejewski JP, Wilmsink JW, van Noorden CJF: **Wild-type and mutated *IDH1/2* enzymes and therapy responses.** *Oncogene* 2018, **37**:1949–1960.
6. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinić-Haberle I, Jones S, Riggins GJ, *et al.*: ***IDH1* and *IDH2* mutations in gliomas.** *N Engl J Med* 2009, **360**:765–773.
7. Medeiros BC, Fathi AT, DiNardo CD, Pollyea DA, Chan SM, Swords R: **Isocitrate dehydrogenase mutations in myeloid malignancies.** *Leukemia* 2017, **31**:272–281.
8. Al-Khallaif H: **Isocitrate dehydrogenases in physiology and cancer: biochemical and molecular insight.** *Cell Biosci* 2017, **7**:37.
9. Gabriel JL, Zervos PR, Plaut GWE: **Activity of purified NAD-specific isocitrate dehydrogenase at modulator and substrate concentrations approximating conditions in mitochondria.** *Metabolism* 1986, **35**:661–667.
10. Reitman ZJ, Yan H: **Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism.** *J Natl Cancer Inst* 2010, **102**:932–941.
11. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, *et al.*: **Cancer-associated *IDH1* mutations produce 2-hydroxyglutarate.** *Nature* 2009, **462**:739–744.
12. Xu X, Zhao J, Xu Z, Peng B, Huang Q, Arnold E, Ding J: **Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity.** *J Biol Chem* 2004, **279**:33946–33957.
13. Yen K, Travins J, Wang F, David MD, Artin E, Straley K, Padyana A, Gross S, DeLaBarre B, Tobin E, *et al.*: **AG-221, a first-in-class therapy targeting acute myeloid leukemia harboring oncogenic *IDH2* mutations.** *Cancer Discov* 2017, **7**: 478–493.
14. Xie X, Baird D, Bowen K, Capka V, Chen J, Chenail G, Cho Y, Dooley J, Farsidjani A, Fortin P, *et al.*: **Allosteric mutant *IDH1* inhibitors reveal mechanisms for *IDH1* mutant and isoform selectivity.** *Structure* 2017, **25**:506–513.
15. Losman JA, Kaelin WG: **What a difference a hydroxyl makes: mutant *IDH*, (*R*)-2-hydroxyglutarate, and cancer.** *Genes Dev* 2013, **27**:836–852.
16. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, *et al.*: **Type and frequency of *IDH1* and *IDH2* mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas.** *Acta Neuropathol* 2009, **118**:469–474.
17. Gupta R, Flanagan S, Li CC, Lee M, Shivalingham B, Maleki S, Wheeler HR, Buckland ME: **Expanding the spectrum of *IDH1* mutations in gliomas.** *Mod Pathol* 2013, **26**:619–625.
18. Bhavya B, Anand CR, Madhusoodanan UK, Rajalakshmi P, Krishnakumar K, Easwer HV, Deepti AN, Gopala S: **To be wild or mutant: role of isocitrate dehydrogenase 1 (*IDH1*) and 2-hydroxy glutarate (2-HG) in gliomagenesis and treatment outcome in glioma.** *Cell Mol Neurobiol* 2020, **40**:53–63.
19. Chotirat S, Thongnoppakhun W, Promsuwicha O, Boonthimat C, Auewarakul CU: **Molecular alterations of isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) metabolic genes and additional genetic mutations in newly diagnosed acute myeloid leukemia patients.** *J Hematol Oncol* 2012, **5**:5.
20. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim S-H, Ito S, Yang C, Wang P, Xiao M-T, *et al.*: **Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α -ketoglutarate-dependent dioxygenases.** *Canc Cell* 2011, **19**:17–30.
21. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA, Cross JR, Fantin VR, Hedvat CV, Perl AE, *et al.*: **The common feature of leukemia-associated *IDH1* and *IDH2* mutations is a neomorphic enzyme activity converting α -ketoglutarate to 2-hydroxyglutarate.** *Canc Cell* 2010, **17**:225–234.
22. Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, Sasaki M, Jin S, Schenkein DP, Su SM, *et al.*: **Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations.** *J Exp Med* 2010, **207**:339–344.
23. Miyata S, Tominaga K, Sakashita E, Urabe M, Onuki Y, Gomi A, Yamaguchi T, Mieno M, Mizukami H, Kume A, *et al.*: **Comprehensive metabolomic analysis of *IDH1*R132H clinical glioma samples reveals suppression of β -oxidation due to carnitine deficiency.** *Sci Rep* 2019, **9**:9787.

Work linking *IDH* mutations to impaired fatty acid β -oxidation due to reduced formation of carnitine / acyl-carnitine, possibly due to D-2HG inhibition of 2OG oxygenases involved in carnitine biosynthesis.

24. Ohka F, Ito M, Ranjit M, Senga T, Motomura A, Motomura K, Saito K, Kato K, Kato Y, Wakabayashi T, *et al.*: **Quantitative metabolome analysis profiles activation of glutaminolysis in glioma with IDH1 mutation.** *Tumor Biol* 2014, **35**:5911–5920.
 25. Lin AP, Abbas S, Kim SW, Ortega M, Bouamar H, Escobedo Y, Varadarajan P, Qin Y, Sudderth J, Schulz E, *et al.*: **D2HGDH regulates alpha-ketoglutarate levels and dioxygenase function by modulating IDH2.** *Nat Commun* 2015, **6**:7768.
 26. Kalinina J, Carroll A, Wang L, Yu Q, Mancheno DE, Wu S, Liu F, Ahn J, He M, Mao H, *et al.*: **Detection of “oncometabolite” 2-hydroxyglutarate by magnetic resonance analysis as a biomarker of IDH1/2 mutations in glioma.** *J Mol Med* 2012, **90**:1161–1171.
 27. Shen X, Voets N, Larkin S, de Pennington N, Plaha P, Stacey R, McCullagh J, Schofield C, Clare S, Jezzard P, *et al.*: **A noninvasive comparison study between human gliomas with IDH1 and IDH2 mutations by MR spectroscopy.** *Metabolites* 2019, **9**:35.
 28. Emir UE, Larkin SJ, de Pennington N, Voets N, Plaha P, Stacey R, Al-Qahtani K, McCullagh J, Schofield CJ, Clare S, *et al.*: **Noninvasive quantification of 2-hydroxyglutarate in human gliomas with IDH1 and IDH2 mutations.** *Cancer Res* 2016, **76**:43–49.
 29. Cardaci S, Ciriolo MR: **TCA cycle defects and cancer: when metabolism tunes redox state.** *Int J Cell Biol* 2012, **2012**:1–9.
 30. Schofield CJ, Ratcliffe PJ: **Oxygen sensing by HIF hydroxylases.** *Nat Rev Mol Cell Biol* 2004, **5**:343–354.
 31. Islam MS, Leissing TM, Chowdhury R, Hopkinson RJ, Schofield CJ: **2-Oxoglutarate-Dependent oxygenases.** *Annu Rev Biochem* 2018, **87**:585–620.
 32. Chowdhury R, Yeoh KK, Tian Y-M, Hillringhaus L, Bagg EA, Rose NR, Leung IKH, Li XS, Woon ECY, Yang M, *et al.*: **The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases.** *EMBO Rep* 2011, **12**:463–469.
 33. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, *et al.*: **IDH mutation impairs histone demethylation and results in a block to cell differentiation.** *Nature* 2012, **483**:474–478.
 34. Schvartzman JM, Reuter VP, Koche RP, Thompson CB: **2-hydroxyglutarate inhibits MyoD-mediated differentiation by preventing H3K9 demethylation.** *Proc Natl Acad Sci* 2019, **116**:12851–12856.
- Work showing IDH2 mutant bearing mesenchymal cells manifest increased H3K9 methylation resulting in impaired transcription factor mediated regulation of cell differentiation.
35. Carboneau M, Gagné L M, Lalonde M-E, Germain M-A, Motorina A, Guiot M-C, Secco B, Vincent EE, Tumber A, Hulea L, *et al.*: **The oncometabolite 2-hydroxyglutarate activates the mTOR signalling pathway.** *Nat Commun* 2016, **7**:12700.
 36. Carey BW, Finley LWS, Cross JR, Allis CD, Thompson CB: **Intracellular α -ketoglutarate maintains the pluripotency of embryonic stem cells.** *Nature* 2015, **518**:413–416.
 37. Yeung BH, Huang J, An SS, Solway J, Mitzner W, Tang W: **Role of isocitrate dehydrogenase 2 on DNA hydroxymethylation in human airway smooth muscle cells.** *Am J Respir Cell Mol Biol* 2020, <https://doi.org/10.1165/rcmb.2019-0323OC>.
 38. Sulkowski PL, Oeck S, Dow J, Economos NG, Mirfakhraie L, Liu Y, Noronha K, Bao X, Li J, Shuch BM, *et al.*: **Oncometabolites suppress DNA repair by disrupting local chromatin signalling.** *Nature* 2020, <https://doi.org/10.1038/s41586-020-2363-0>.
- Detailed study linking elevated small-molecule oncometabolite (2HG, succinate, fumarate) levels with DNA damage repair via JmJC KDM inhibition.
39. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, *et al.*: **Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation.** *Canc Cell* 2010, **18**:553–567.
 40. Sasaki M, Knobbe CB, Itsumi M, Elia AJ, Harris IS, Chio IIC, Cairns RA, McCracken S, Wakeham A, Haight J, *et al.*: **d-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function.** *Genes Dev* 2012, **26**:2038–2049.
 41. Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, *et al.*: **Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation.** *Nature* 2012, **483**:484–488.
 42. Wang P, Wu J, Ma S, Zhang L, Yao J, Hoadley KA, Wilkerson MD, Perou CM, Guan K-L, Ye D, *et al.*: **Oncometabolite d-2-hydroxyglutarate inhibits ALKBH DNA repair enzymes and sensitizes IDH mutant cells to alkylating agents.** *Cell Rep* 2015, **13**:2353–2361.
 43. Chen F, Bian K, Tang Q, Fedele BI, Singh V, Humulock ZT, Essigmann JM, Li D: **Oncometabolites d- and l-2-hydroxyglutarate inhibit the AlkB family DNA repair enzymes under physiological conditions.** *Chem Res Toxicol* 2017, **30**:1102–1110.
 44. Elkashef SM, Lin A-P, Myers J, Sill H, Jiang D, Dahia PLM, Aguiar RCT: **IDH mutation, competitive inhibition of FTO, and RNA methylation.** *Canc Cell* 2017, **31**:619–620.
 45. Su R, Dong L, Li C, Nachtergaele S, Wunderlich M, Qing Y, Deng X, Wang Y, Weng X, Hu C, *et al.*: **R-2HG exhibits anti-tumor activity by targeting FTO/m6A/MYC/CEBPA signaling.** *Cell* 2018, **172**:90–105.e23.
- Work showing d-2HG can exert anti-leukaemic activity in a manner linked to altered RNA N⁶-methyl adenosine status via inhibition of the fat mass and obesity protein (FTO) nicely illustrating context dependent effects of d-2HG.
46. Fu X, Chin RM, Vergnes L, Hwang H, Deng G, Xing Y, Pai MY, Li S, Ta L, Fazlollahi F, *et al.*: **2-Hydroxyglutarate inhibits ATP synthase and mTOR signaling.** *Cell Metab* 2015, **22**:508–515.
 47. Yang Z, Jiang B, Wang Y, Ni H, Zhang J, Xia J, Shi M, Hung L-M, Ruan J, Mak TW, *et al.*: **2-HG inhibits necroptosis by stimulating DNMT1-dependent hypermethylation of the RIP3 promoter.** *Cell Rep* 2017, **19**:1846–1857.
 48. McBrayer SK, Mayers JR, DiNatale GJ, Shi DD, Khanal J, Chakraborty AA, Sarosiek KA, Briggs KJ, Robbins AK, Sewastianik T, *et al.*: **Transaminase inhibition by 2-hydroxyglutarate impairs glutamate biosynthesis and redox homeostasis in glioma.** *Cell* 2018, **175**:101–116.e25.
- Work linking 2OG dependent transaminase inhibition and altered metabolism in IDH1 mutant bearing cells.
49. Chen Z, Zang J, Whetstone J, Hong X, Davrazou F, Kutateladze TG, Simpson M, Mao Q, Pan C-H, Dai S, *et al.*: **Structural insights into histone demethylation by JMJD2 family members.** *Cell* 2006, **125**:691–702.
 50. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AWM, Lu C, Ward PS, *et al.*: **IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype.** *Nature* 2012, **483**:479–483.
 51. Oberley LW, Oberley TD, Buettner GR: **Cell differentiation, aging and cancer: the possible roles of superoxide and superoxide dismutases.** *Med Hypotheses* 1980, **6**:249–268.
 52. Mills E, O'Neill LAJ: **Succinate: a metabolic signal in inflammation.** *Trends Cell Biol* 2014, **24**:313–320.
 53. Webb JD, Coleman ML, Pugh CW: **Hypoxia, hypoxia-inducible factors (HIF), HIF hydroxylases and oxygen sensing.** *Cell Mol Life Sci* 2009, **66**:3539–3554.
 54. Metellus P, Colin C, Taieb D, Guedj E, Nanni-Metellus I, de Paula AM, Colavolpe C, Fuentes S, Dufour H, Barrie M, *et al.*: **IDH mutation status impact on *in vivo* hypoxia biomarkers expression: new insights from a clinical, nuclear imaging and immunohistochemical study in 33 glioma patients.** *J Neuro Oncol* 2011, **105**:591–600.
 55. Williams SC, Karajannis MA, Chiriboga L, Golfinos JG, von Deimling A, Zagzag D: **R132H-mutation of isocitrate dehydrogenase-1 is not sufficient for HIF-1 α upregulation in adult glioma.** *Acta Neuropathol* 2011, **121**:279–281.
 56. Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, *et al.*: **Glioma-derived mutations in IDH1**

- dominantly inhibit IDH1 catalytic activity and induce HIF-1 α . *Science* (80-) 2009, **324**:261–265.
57. Das S, Marsden PA: **Angiogenesis in glioblastoma.** *N Engl J Med* 2013, **369**:1561–1563.
 58. Tarhonskaya H, Rydzik AM, Leung IKH, Loik ND, Chan MC, Kawamura A, McCullagh JSO, Claridge TDW, Flashman E, Schofield CJ: **Non-enzymatic chemistry enables 2-hydroxyglutarate-mediated activation of 2-oxoglutarate oxygenases.** *Nat Commun* 2014, **5**:3423.
 59. Yu T, Tang B, Sun X: **Development of inhibitors targeting hypoxia-inducible factor 1 and 2 for cancer therapy.** *Yonsei Med J* 2017, **58**:489.
 60. Acker T, Diez-Juan A, Aragones J, Tjwa M, Brusselmans K, Moons L, Fukumura D, Moreno-Murciano MP, Herbert J-M, Burger A, *et al.*: **Genetic evidence for a tumor suppressor role of HIF-2 α .** *Canc Cell* 2005, **8**:131–141.
 61. Song LP, Zhang J, Wu S-F, Huang Y, Zhao Q, Cao J-P, Wu Y-L, Wang L-S, Chen G-Q: **Hypoxia-inducible factor-1 α -induced differentiation of myeloid leukemic cells is its transcriptional activity independent.** *Oncogene* 2008, **27**:519–527.
 62. Kickingereder P, Sahm F, Radbruch A, Wick W, Heiland S, Deimling A von, Bendszus M, Wiestler B: **IDH mutation status is associated with a distinct hypoxia/angiogenesis transcriptome signature which is non-invasively predictable with rCBV imaging in human glioma.** *Sci Rep* 2015, **5**:16238.
 63. Jo SH, Son M-K, Koh H-J, Lee S-M, Song I-H, Kim Y-O, Lee Y-S, Jeong K-S, Kim WB, Park J-W, *et al.*: **Control of mitochondrial redox balance and cellular defense against oxidative damage by mitochondrial NADP⁺-dependent isocitrate dehydrogenase.** *J Biol Chem* 2001, **276**:16168–16176.
 64. Bleeker FE, Atai NA, Lamba S, Jonker A, Rijkeboer D, Bosch KS, Tigchelaar W, Troost D, Vandertop WP, Bardelli A, *et al.*: **The prognostic IDH1 R132 mutation is associated with reduced NADP⁺-dependent IDH activity in glioblastoma.** *Acta Neuropathol* 2010, **119**:487–494.
 65. Shi J, Sun B, Shi W, Zuo H, Cui D, Ni L, Chen J: **Decreasing GSH and increasing ROS in chemosensitivity gliomas with IDH1 mutation.** *Tumor Biol* 2015, **36**:655–662.
 66. Gelman SJ, Naser F, Mahieu NG, McKenzie LD, Dunn GP, Chheda MG, Patti GJ: **Consumption of NADPH for 2-HG synthesis increases pentose phosphate pathway flux and sensitizes cells to oxidative stress.** *Cell Rep* 2018, **22**:512–522.
 67. Hollinshead KER, Munford H, Eales KL, Bardella C, Li C, Escribano-Gonzalez C, Thakker A, Nonnenmacher Y, Kluckova K, Jeeves M, *et al.*: **Oncogenic IDH1 mutations promote enhanced proline synthesis through PYCR1 to support the maintenance of mitochondrial redox homeostasis.** *Cell Rep* 2018, **22**:3107–3114.
 68. Mayers JR, Vander Heiden MG: **BCAT1 defines gliomas by IDH status.** *Nat Med* 2013, **19**:816–817.
 69. Golub D, Iyengar N, Dogra S, Wong T, Bready D, Tang K, Modrek AS, Placantonakis DG: **Mutant isocitrate dehydrogenase inhibitors as targeted cancer therapeutics.** *Front Oncol* 2019, **9**.
 70. Okoye-Okafor UC, Bartholdy B, Cartier J, Gao EN, Pietrak B, Rendina AR, Rominger C, Quinn C, Smallwood A, Wiggall KJ, *et al.*: **New IDH1 mutant inhibitors for treatment of acute myeloid leukemia.** *Nat Chem Biol* 2015, **11**:878–886.
 71. Pusch S, Krausert S, Fischer V, Balss J, Ott M, Schrimpf D, Capper D, Sahm F, Eisel J, Beck A-C, *et al.*: **Pan-mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma in vivo.** *Acta Neuropathol* 2017, **133**:629–644.
 72. Merk A, Bartesaghi A, Banerjee S, Falconieri V, Rao P, Davis MI, Pragani R, Boxer MB, Earl LA, Milne JLS, *et al.*: **Breaking cryo-EM resolution barriers to facilitate drug discovery.** *Cell* 2016, **165**:1698–1707.
 73. Cho YS, Levell JR, Liu G, Caferro T, Sutton J, Shafer CM, Costales A, Manning JR, Zhao Q, Sendzik M, *et al.*: **Discovery and evaluation of clinical candidate IDH305, a brain penetrant mutant IDH1 inhibitor.** *ACS Med Chem Lett* 2017, **8**:1116–1121.
 74. Deng G, Shen J, Yin M, McManus J, Mathieu M, Gee P, He T, Shi C, Bedel O, McLean LR, *et al.*: **Selective inhibition of mutant isocitrate dehydrogenase 1 (IDH1) via disruption of a metal binding network by an allosteric small molecule.** *J Biol Chem* 2015, **290**:762–774.
 75. Ma R, Yun CHH: **Crystal structures of pan-IDH inhibitor AG-881 in complex with mutant human IDH1 and IDH2.** *Biochem Biophys Res Commun* 2018, **503**:2912–2917.
 76. Caravella JA, Lin J, Diebold RB, Campbell A-M, Ericsson A, Gustafson G, Wang Z, Castro J, Clarke A, Gotur D, *et al.*: **Structure-Based design and identification of FT-2102 (olutasidenib), a potent mutant-selective IDH1 inhibitor.** *J Med Chem* 2020, **63**:1612–1623.
 77. Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, Straley K, Kerytsky A, Liu W, Gliser C, *et al.*: **Targeted inhibition of mutant IDH2 in leukemic cells induces cellular differentiation.** *Science* 2013, **340**:622–626.
 78. FDA: **FDA approves ivosidenib as first-line treatment for AML with IDH1 mutation.** 2019.
 79. FDA: **FDA granted regular approval to enasidenib for the treatment of relapsed or refractory AML.** 2017.
 80. Konteatis Z, Artin E, Nicolay B, Straley K, Padyana AK, Jin L, Chen Y, Narayanaswamy R, Tong S, Wang F, *et al.*: **Vorasidenib (AG-881): a first-in-class, brain-penetrant dual inhibitor of mutant IDH1 and 2 for treatment of glioma.** *ACS Med Chem Lett* 2020, **11**:101–107.
- Description of the first brain penetrant inhibitor of both IDH1/IDH2 variants suitable for evaluation in glioma treatment.
81. DiNardo CD, Stein EM: **SOHO state of the art update and next questions: IDH therapeutic targeting in AML.** *Clin Lymphoma Myeloma Leuk* 2018, **18**:769–772.
 82. Popovici-Muller J, Lemieux RM, Artin E, Saunders JO, Salituro FG, Travins J, Cianchetta G, Cai Z, Zhou D, Cui D, *et al.*: **Discovery of AG-120 (ivosidenib): a first-in-class mutant IDH1 inhibitor for the treatment of IDH1 mutant cancers.** *ACS Med Chem Lett* 2018, **9**:300–305.
 83. Urban DJ, Martinez NJ, Davis MI, Brimacombe KR, Cheff DM, Lee TD, Henderson MJ, Titus SA, Pragani R, Rohde JM, *et al.*: **Assessing inhibitors of mutant isocitrate dehydrogenase using a suite of pre-clinical discovery assays.** *Sci Rep* 2017, **7**:12758.
 84. Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komisopoulou E, *et al.*: **An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells.** *Science* (80-) 2013, **340**:626–630.
 85. Popovici-Muller J, Saunders JO, Salituro FG, Travins JM, Yan S, Zhao F, Gross S, Dang L, Yen KE, Yang H, *et al.*: **Discovery of the first potent inhibitors of mutant IDH1 that lower tumor 2-HG in vivo.** *ACS Med Chem Lett* 2012, **3**:850–855.
 86. Davis MI, Gross S, Shen M, Straley KS, Pragani R, Lea WA, Popovici-Muller J, DeLaBarre B, Artin E, Thorne N, *et al.*: **Biochemical, cellular, and biophysical characterization of a potent inhibitor of mutant isocitrate dehydrogenase IDH1.** *J Biol Chem* 2014, **289**:13717–13725.
 87. Chaturvedi A, Herbst L, Pusch S, Klett L, Goparaju R, Stichel D, Kaulfuss S, Panknin O, Zimmermann K, Toschi L, *et al.*: **Pan-mutant-IDH1 inhibitor BAY1436032 is highly effective against human IDH1 mutant acute myeloid leukemia in vivo.** *Leukemia* 2017, **31**:2020–2028.
 88. Levell JR, Caferro T, Chenail G, Dix I, Dooley J, Firestone B, Fortin PD, Giraldez J, Gould T, Gowney JD, *et al.*: **Optimization of 3-Pyrimidin-4-yl-oxazolidin-2-ones as allosteric and mutant specific inhibitors of IDH1.** *ACS Med Chem Lett* 2017, **8**: 151–156.
 89. FDA Centre for drug evaluation and research: **209606Orig1s000 Multi-discipline review.** 2017.
 90. Intlekofer AM, Shih AH, Wang B, Nazir A, Rustenburg AS, Albanese SK, Patel M, Famulare C, Correa FM, Takemoto N, *et al.*: **Acquired resistance to IDH inhibition through trans or cis dimer-interface mutations.** *Nature* 2018, **559**:125–129.

Resistance to the 'allosteric' inhibitors, ivosidenib and enasidenib, is shown to be via mutations at the IDH1 and IDH2 variant dimer interface respectively.

91. Jin G, Reitman ZJ, Duncan CG, Spasojevic I, Gooden DM, Rasheed BA, Yang R, Lopez GY, He Y, McLendon RE, *et al.*: **Disruption of wild-type IDH1 suppresses d-2-hydroxyglutarate production in IDH1-mutated gliomas.** *Cancer Res* 2013, **73**:496–501.
92. Anderson NM, Li D, Peng HL, Laroche FJF, Mansour MR, Gjini E, Aioub M, Helman DJ, Roderick JE, Cheng T, *et al.*: **The TCA cycle transferase DLST is important for MYC-mediated leukemogenesis.** *Leukemia* 2016, **30**:1365–1374.
93. Thompson MG, Blake-Hedges JM, Cruz-Morales P, Barajas JF, Curran SC, Eiben CB, Harris NC, Benites VT, Gin JW, Sharpless WA, *et al.*: **Massively parallel fitness profiling reveals multiple novel enzymes in *Pseudomonas putida* lysine metabolism.** *MBio* 2019, **10**. e02577-18.
94. Wang P, Dong Q, Zhang C, Kuan P-F, Liu Y, Jeck WR, Andersen JB, Jiang W, Savich GL, Tan T-X, *et al.*: **Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas.** *Oncogene* 2013, **32**:3091–3100.
95. Núñez FJ, Mendez FM, Kadiyala P, Alghamri MS, Savelieff MG, Garcia-Fabiani MB, Haase S, Koschmann C, Calinescu A-A, Kamran N, *et al.*: **IDH1-R132H acts as a tumor suppressor in glioma via epigenetic up-regulation of the DNA damage response.** *Sci Transl Med* 2019, **11**, eaaq1427.
96. Amankulor NM, Kim Y, Arora S, Kargl J, Szulzewsky F, Hanke M, Margineantu DH, Rao A, Bolouri H, Delrow J, *et al.*: **Mutant IDH1 regulates the tumor-associated immune system in gliomas.** *Genes Dev* 2017, **31**:774–786.
97. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, Morozova O, Newton Y, Radenbaugh A, Pagnotta SM, *et al.*: **Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma.** *Cell* 2016, **164**:550–563.
98. Tran AN, Lai A, Li S, Pope WB, Teixeira S, Harris RJ, Woodworth DC, Nghiemphu PL, Cloughesy TF, Ellingson BM: **Increased sensitivity to radiochemotherapy in IDH1 mutant glioblastoma as demonstrated by serial quantitative MR volumetry.** *Neuro Oncol* 2014, **16**:414–420.